ISSN 2449-8955 European Journal of Biological Research *Review Article*

DOI: http://dx.doi.org/10.5281/zenodo.5091854

Tissue culture approaches to improve nutritional quality and stress response in peanut

Amit Das [1,2], Juran C. Goyali [3], Aleya Ferdausi [1*]

[1] Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

[2] Gafargaon Islamia Government High School, Gafargaon, Mymensingh, Bangladesh

[3] Centre for Aquaculture and Seafood Development, Fisheries and Marine Institute of Memorial University of Newfoundland, St. John's, NL, Canada

* Corresponding author: Phone: +8801747173836, Email: aferdausi.gpb@bau.edu.bd

Received: 29 April 2021; **Revised submission:** 25 May 2021; **Accepted:** 08 July 2021

https://jbrodka.com/index.php/ejbr

ABSTRACT: Peanut, also known as groundnut (*Arachis hypogaea* L.), is an annual leguminous oil crop cultivated worldwide for food and fodder. Several stress factors critically diminish the productivity and nutritional quality of this protein-rich plant. *In vitro* cell and tissue culture systems have been used in many plant species to rapidly propagate large numbers of plants, create somaclonal variation, produce bioactive compounds, and enable genetic engineering. Tissue culture based mutagenesis and genetic engineering are particularly attractive for crop improvement. Tissue culture techniques have been implicated over the years to improve peanut, despite the general recalcitrant nature of this species to *in vitro* culture. In this manuscript, we review the progress that has been made on *in vitro* culture of peanut, and its application to improve nutritional quality and resistance to major biotic and abiotic stresses in peanut.

Keywords: Peanut; *In vitro*; Genetic transformation; Biotic and abiotic stresses; Nutritional quality.

Abbreviations: INDELS – insertions and deletions; MS – Murashige and Skoog; B5 – Gamborg medium; BAP – benzyl amino purine; Kn – kinetin, TDZ – thidiazuron; 2,4-D – 2,4-dichlorodiphenoxyacetic acid, NAA – naphthalene acetic acid, IBA – indole butyric acid, IAA – indole acetic acid; FAD – fatty acid desaturase; TALENs – transcription activator like effector nucleases; ELISA – enzyme-linked immunosorbent assay; ACC – 1-aminocyclopropane-1-carboxylic acid.

1. INTRODUCTION

Peanut (*Arachis hypogaea* L.; family Fabaceae and subfamily Faboideae) is an important leguminous food and cash crop originally from South America. Peanut is cultivated throughout Asia, Europe, Africa, Oceania, North and South America in tropical, sub-tropical and warm temperature areas [1]. It is the fourth most important oil crop in the world after soybean, oilseed rape, and cotton [2, 3].

Globally 27.7 million hectares (ha) area is under peanut cultivation with a total annual production of 44.0 million tons; led by China (37.9%), India (15.6%), Nigeria (6.9%) and the United States (5.9%) [4]. In Bangladesh, peanut is the third most important oilseed crop after mustard and sesame in terms of production [5]. Usually, 41% of globally produced peanut is used as food and 49% is processed for the extraction of edible oil.

The oil cake meal, which remains after oil extraction is widely used for industrial purposes and livestock feed, contains up to 50% protein [6]. Besides edible oil production, peanut contains proteins, fibre, vitamins, minerals and essential amino acids, which can be added as functional ingredients into many processed foods [7-9]. Peanut is a substantial source of bioactive compounds like polyphenols, resveratrol, phenolic acids, flavonoids and phytosterols that block the absorption of cholesterol from the diet as well as possess disease prevention properties [8, 9].

Peanut is sensitive to numerous biotic and abiotic stresses, including insect pest infestation, salinity, drought, and high temperatures, leading to major yield and quality losses [2, 10, 11]. In Bangladesh, salinity and drought are the major abiotic factors drastically affecting peanut yield and seed quality [12, 13]. Aflatoxigenic fungi produce aflatoxins in peanut. Aflatoxin contamination can be enhanced under drought conditions, consequently making the peanut unfit for human consumption [12, 14, 15]. Diseases, such as stem rot, collar rot, seedling blight, peanut tikka late leaf spot disease, dry wilt, afla root, leaf spots, rust and bud necrosis cause economic losses in peanut production due to crop failure or deterioration of pod quality [10]. Besides diseases, different insects also incur major economic loss in peanut. Aphids are the most disparaging insect vectors of peanut rosette disease, a major viral disease that causes severe reductions in yield and quality [1, 16].

Cell and tissue culture techniques are widely used in peanut to improve nutritional quality including higher yield and tolerance to biotic and abiotic stresses [15, 17]. Explant culture in an appropriate medium often results in an unorganized and dividing mass of cells named callus [18, 19]. Differentiation of the callus, results in the production of bioactive compounds such as polyamines and osmolytes, glycine, proline, betaine, which mainly serve in defense against biotic and abiotic stresses [19-22]. Callus induction followed by plant regeneration can induce genetic and epigenetic changes causing somaclonal variation [18, 23, 24]. The application of in vitro cell or tissue culture and development of somaclonal variations is reported in groundnut crop improvement worldwide [15, 17, 25-28]. Conversely, the commercial applications of somaclonal variations are still far from complete. However, knowledge on the developmental and molecular basis of this variation could be a prerequisite to develop groundnut genotypes with high yield, biotic and abiotic stress tolerance, active metabolite production, nutritional improvement, and crop quality.

This review represents an overview of *in vitro* practices and obstacles for peanut regeneration, and the application of tissue culture approaches to improve the nutritional quality and stress tolerance ability of peanut.

2. NUTRITIONAL VALUE OF PEANUT

A healthy population is an indispensable requirement to promote development in any country, and better nutrition is a fundamental human right. Therefore, the relation between food, nutrition and health should be reinforced. The consumption of either raw or processed peanuts is beneficial to human health because of their high nutrient content, including protein, fat, fibre, minerals and vitamins [7, 9] (Table 1).

The peanut contains plant-based protein, including all essential amino acids. It comprises of unsaturated fatty acids like monounsaturated and paraformaldehyde fatty acids which are heart friendly [9, 29, 30]. Furthermore, peanut is considered as a functional food due to the presence of Coenzyme Q10, which is mandatory to cure cardiovascular diseases [7, 9]. Peanut is an abundant source of vitamins like niacin, folate, thiamin, riboflavin, pantothenic acid, pyridoxine and vitamin E [9]. Additionally, it is a good source of minerals such as iron, zinc, potassium and magnesium, including antioxidant minerals like selenium, manganese and copper [9]. These vitamins and minerals play important functioning roles in the digestive systems, skin, nerves, and also reduce inflammation and risk of metabolic syndrome [31-35]. It also contains

antioxidants and bioactive compounds such as flavonoids, resveratrol, chlorogenic acid, caffeic acid, coumaric acid, ferulic acid and stilbene that are well-known for their disease preventative properties [9, 36-38]. Phytosterols and resveratrol in peanut have been reported to reduce the growth of prostate, colon and rectal cancer cells [39-41]. Peanut consumption also protects against type II diabetes [42, 43] and obesity [7, 35]. Vitamins in peanut like niacin and vitamin E have a protective effect against early to mid-stage Alzheimer's disease [31]. Iron and zinc are widely reported to combat malnutrition and anemia, especially in women and children in Asia and Africa, which could be supplemented through peanut consumption [8]. Besides raw and roasted peanuts, products such as peanut butter and oil are also beneficial to heart health through reducing cholesterol levels [9]. Fresh peanuts and fermented peanut meal exhibited antioxidant properties through scavenging free radicals generated in the human body [36, 44, 45].

Table 1. Nutritional value of peanut (*Arachis hypogaea* L.) per 100 gram.

Component	**Nutrient value**	**References**
Free energy	567 Kcal	[8, 9, 30]
Carbohydrates	16-20 g	[7, 8, 9, 30]
Protein	25-28 g	[7, 8, 9, 30, 114]
Total Fat	49.2 g	[7, 8, 30]
Dietary Fiber	8.5 g	[7, 8, 30]
Tannin	38.0 g	[114]
Edible oil	48-60 g	[8, 115]
Minerals	**Nutrient value (mg)**	**References**
Sodium	18	[9, 30]
Potassium	705	[9, 30]
Magnesium	168	[9, 30]
Calcium	92	[9, 30]
Iron	4.6-6.8	[8, 9, 30]
Zinc	3.3-9.5	[8, 9, 30]
Phosphorus	76	[9, 30]
Copper	1.2	[9, 30]
Manganese	1.9	[9, 30]
Selenium	3.3 µg	[9, 30]
Vitamins	**Nutrient value (mg)**	**References**
Folates	0.2	[9, 30]
Niacin	12.1-16.0	[7, 9, 30]
Pantothenic acid	1.8	[9, 30]
Pyridoxine	0.4	[9, 30]
Riboflavin	0.1	[9, 30]
Thiamin	0.7-1.0	[7]
Tocopherol	18.6-21.1	[7]
β-carotene	63.3-65.4	[7]

3. INDUCED MOLECULAR CHANGES IN PLANT *IN VITRO* CULTURE

Plant tissue culture enhances the inherent variability, which leads to mutations or variations in high frequency that could be the narrative source of genetic variability in plants [46]. These mutations often exhibit phenotypic variation named somaclonal variation, which induces stable genetic or epigenetic variations in the regenerated plants [47, 48]. Such variations are considered as a major drawback of tissue culture in commercial micropopagation to achieve true to type population. However, somaclonal variations could be exploited in peanut crop improvement [47, 49, 50].

In vitro culture is assumed to generate the changes in chromosomal and DNA sequence, protein expression, metabolite content, DNA methylation, and transposon activation, chromatin remodelling, small RNA mediated regulation leading to somaclonal variations [48, 51-53]. *In vitro* growth environment is accompanied by permanent genetic changes leading to significant genome alterations through changes in chromosomal level [51]. Chromosome structural changes, i.e., breakage and rearrangements, occur more than numerical changes in regenerated plants [23, 51]. DNA sequence variations such as single base pair changes, single nucleotide substitution mutation, deamination and small INDELS are major molecular changes often reported in cultured tissues [54, 55]. Ribosomal DNA repeats, DNA microsatellites and transposable elements are extremely sensitive to stress conditions, and the major sources of mutations or variations occur during cell culture [56]. Epigenetic gene expression includes heritable, reversible and enzyme arbitrated chemical modifications to the DNA and associated proteins, which is reflected as an alteration in DNA methylation, chromatin remodelling and small RNA mediated regulation [23, 57]. Therefore, *in vitro* tissue culture technique creates molecular changes, which regulate the physiological, biochemical and molecular aspects of plant development and stress response.

4. PEANUT TISSUE CULTURE

The biotechnological and molecular breeding techniques of crop improvement exclusively rely on the establishment of persistent, efficient and rapid *in vitro* regeneration systems for commercial applications [48, 58].

4.1. Major obstacles in peanut tissue culture

Peanut tissue culture is extremely challenging due to its highly recalcitrant nature, and *in vitro* regeneration success is very low [59-61]. Peanut tissue culture has been reported as a suitable protocol for genetic transformation. However, low regeneration coupled with prevailing sterility associated with regenerated plants is a major constraint for genetic transformation [27, 62-64]. *In vitro* organogenesis through callus differentiation and morphogenesis is more suitable for the application of biotechnological and molecular strategies for crop improvement than direct somatic embryogenesis although the latter method has always been indispensable due to its shorter duration in plantlet development [46, 47, 63, 65].

The recalcitrant nature of peanut seed is a major problem for seed storage through drying or freezing. Therefore seed viability often challenges seed germination *in vitro* [66, 67]. Seed's recalcitrant nature varies greatly among the peanut genotypes. The higher recalcitrant nature of seeds is associated with lower transformation efficacy and higher susceptibility to genetic transformation [68]. The embryonic axes and seeds without seed coat significantly exhibited a higher germination rate than the seeds with seed coat [69, 70]. The low germination rate in seeds with seed coat could be associated with the mechanical constraint by the seed coat and the impermeability of water and oxygen required for seed germination [71, 72]. Effective plant regeneration through tissue culture relies on several factors such as appropriate growth media, plant growth regulators,

explants, genotypes, growth environment, including photoperiod, temperature, and humidity [73]. Furthermore, the *Agrobacterium* and micro projectile bombardment mediated gene transfer have been equally exploited in peanut transformation, but the prior method is completely relying on *Agrobacterium*-host compatibility [68]. Therefore, the genetic transformation efficiency is closely linked to tissue culture protocols for transgenic development, selection of suitable explants, and age of cell lines, transgene expressions, and molecular confirmation of their expressions. Hence, the low transformation efficiency cannot be kept aside while choosing a genetic transformation protocol in peanut [68].

4.2. Selection of suitable explant

Cotyledonary nodes, cotyledon, epicotyl, hypocotyl, leaf discs, shoot tips (Figure 1a) are widely used explants for peanut tissue culture that showed sufficient regeneration success *in vitro* [18, 26, 66, 74-76]. Among all these explants the epicotyl and hypocotyl showed better performances in callus induction (Figure 1b) and plant regeneration (Table 2) which could be associated with the presence of meristematic cells near the cut surface of those explants [46, 47, 76]. However, cotyledon explants are mostly preferred in genetic transformation but associated with longer duration and low regeneration frequencies compared to other explants [77].

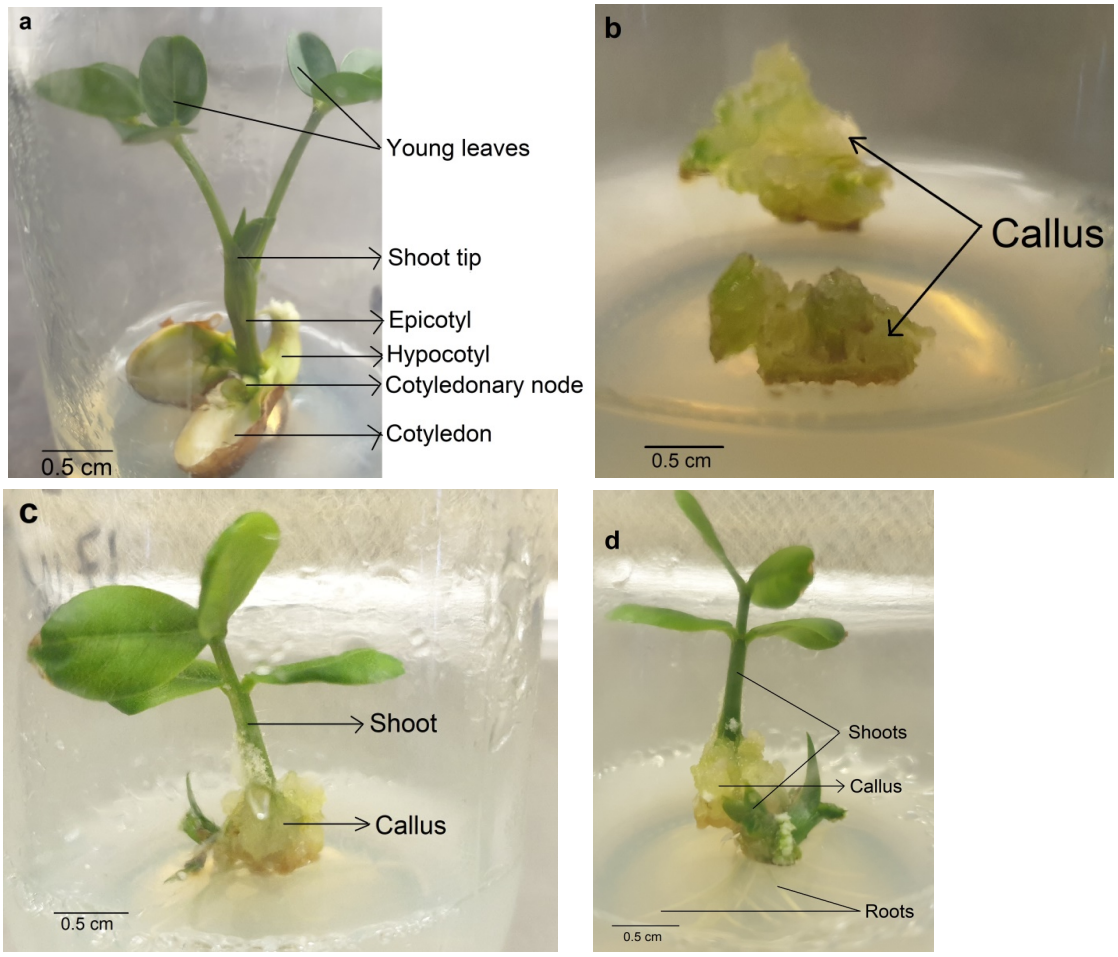

Figure 1. Different stages of peanut plant regeneration under *in vitro* culture; a) small plantlet germinated from seed on MS media showing different explants; b) callus induction from epicotyl explants c) shoot initiation from callus and d) small plantlet with shoots and roots. Scale bars: 0.5 cm. Photographs are taken from the on-going peanut project funded by TWAS.

Table 2. Performance of explant and media composition in peanut (*Arachis hypogaea* L.) plant regeneration under *in vitro* growth condition.

Explant	Media composition (MS media supplemented with growth regulators)			References
	Callus induction	Shoot initiation	Root formation	
Cotyledon (whole), Hypocotyl, Epicotyl, Leaflet	2.0 mg/l 2,4-D 2.0 mg/l NAA	2.0-3.0 mg/l BAP	½ MS + 0.2 mg/l IBA	[76, 116]
Embryo	1.5 mg/l NAA + 5.5 mg/l BAP	5.0 mg/l BAP + 1.5 mg/l TDZ 4.0 mg/l BAP + 1.0 mg/l NAA	1.5 mg/l IBA	[18]
Immature cotyledon	1.5-2.0 mg/l 2, 4-D 1.0-1.5 mg/l BAP 1.5 mg/l 2,4-D + 0.5 mg/l BAP 1.5 mg/l 2, 4-D + 0.5 mg/l Kn	1.0 mg/l BAP + 0.5 mg/l IAA 1.0 mg/l BAP + 1.0 mg/l IAA 0.5 mg/l BAP + 0.5 mg/l 2,4-D 1.5 mg/l BAP + 1.0 mg/l NAA	1.0 mg/l BAP + 1.5 mg/l IAA + ½ MS + 0.5 mg/l IBA	[46, 117, 118]
De-embryonated Cotyledon	0.1 mg/l NAA 1.0 mg/l NAA + 2.0 mg/l BAP	0.1 mg/l NAA + 2.0 mg/l BAP 0.1 mg/l NAA + 4.0 mg/l BAP	B5 + 2.0 mg/l NAA	[64]
Epicotyl, Immature leaves, Hypocotyl, Cotyledon	1.0-3.0 mg/l NAA + 1.0-3.0 mg/l BAP	0.1-0.5 mg/l NAA + 1.0-4.0 mg/l BAP	1.0 mg/l NAA	[47]
Cotyledonary nodes	3.0 mg/l 2,4-D + B5 + 4/5 mg/l NAA	0.15 mg/L BAP + 0.20 mg/L IAA + B5	0.3 mg/l NAA	[61, 77, 90]
De-embryonated cotyledon	3/5 mg/l BAP; 3/5 mg/l Kn 2.0 mg/l BAP + 5.0 mg/l Kn	2.0 mg/l BAP	0.5 mg/l IAA	[24, 74]
Mature and immature Cotyledon, Embryo axes, Epicotyl, Mature and immature embryo, Young leaflets, Leaflet segments	Wide range of growth regulators i.e. 2, 4-D, TDZ, NAA, BAP, Picloram; 3-7 mg/l 2, 4-D showed the best response	4.0 mg/l BAP + 2.0 mg/l NAA		[62]
Leaf discs		0.5 mg/l NAA + 0.5 mg/l TDZ 8 mg/l BAP + 0.5 mg/l NAA	0.5 mg/ NAA	[63, 89]
Cotyledon		4.5 mg/L BAP + 1.0-1.5 mg/L 2,4-D	1.0 mg/l NAA	[91, 119]
Cotyledonary node		5.0 mg/l BAP	0.5 mg/l NAA	[66]
Embryonic leaflets	10 mg/l 2, 4-D	4 mg/l BAP	MS basal media	[96, 120]

½ MS = half strength of MS basal media.

4.3. Media selection

The concentration of ammonia, nitrate, inorganic nutrient and vitamins are higher in MS medium than other growth media such as B5, Lloyd and McCown Woody Plant medium, Schenk and Hildebrandt basal salt medium, hence showed better performance in peanut [24, 78, 79]. Moreover, sugar source i.e. sucrose, glucose, fructose, maltose etc. in media plays a vital role in *in vitro* culture of peanut. Among the sugar sources, sucrose exhibited the best performance in terms of callus induction, shoot initiation and bud regeneration [24]. MS media supplemented with 3% sucrose is optimum for higher multiplication rate in peanut tissue culture, whereas the higher concentration causes tissue necrosis due to a sharp decline in osmotic potential leading to increased phenols. Furthermore, a lower concentration is accompanied with slow growth and multiplication rate [24, 80, 81].

4.4. Selection of growth regulators

Plant regeneration usually depends on the appropriate concentrations and combinations of plant growth regulators. Generally, cytokinins such as benzyl amino purine (BAP), kinetin (Kn), thidiazuron (TDZ) promote

shoot initiation, whereas the auxins such as 2,4-dichlorodiphenoxyacetic acid (2,4-D), naphthalene acetic acid (NAA), indole butyric acid (IBA), indole acetic acid (IAA), picloram induce callus and somatic embryos in peanut [46, 82] (Table 2). High cytokinin combined with low auxin often induces better shoot initiation (Figure 1c). In peanut, maximum number of shoots per explant were observed in a media combination of 6 mg/l BAP + 0.1 mg/l NAA [64] and 5.5 mg/l BAP +1.5 mg/l NAA [18]. BAP and TDZ are the best-studied cytokinins for effective shoot induction in numerous leguminous plants including peanut [83-85]. However, the excessively high concentrations of cytokinins such as 7-10 mg/l BAP or TDZ showed abnormal enlarged tissues during shoot organogenesis [63, 86]. The application of picloram showed better somatic embryogenesis in peanut in compared to 2,4-D [61, 87]. On the contrary, the supplementation of 1-3 mg/l 2,4-D in culture media showed the better callus induction in peanut than with NAA [46, 47, 61, 88]. The high concentrations of BAP and NAA often inhibit the callus induction and different concentrations of NAA showed slow callus growth in peanut [18, 76]. In most of the previous studies, it was observed that the NAA (0.1-2 mg/l) and IBA (0.5-2 mg/l) performed well in root initiation (Figure 1d) than other auxins [18, 46, 61, 63, 66, 89-91]. Yet, a higher concentration of NAA showed malformed callus at the vase of shoot rather than roots [61].

Therefore, it can be assumed that MS medium supplemented with low concentrations of 2,4-D could be used for callus induction. Whereas a higher concentration of BAP combined with low auxins would be more suitable for shoot initiation and organogenesis as compare to 2,4-D and NAA in peanut tissue culture.

5. PEANUT IMPROVEMENT THROUGH TISSUE CULTURE APPROACHES

5.1. Improvement of nutritional quality

Peanut, a functional food, is cultivated over the world for its quality oil, energy, nutrition-rich food and fodder [6]. The quality parameters considered for peanut improvement are high protein, sugar, oil and oleic/linoleic fatty acid ratio, resistance to aflatoxin contamination and allergen [18, 92-94]. Furthermore, organic matter digestibility, metabolizable energy, nitrogen and protein content of haulms are the targeted quality traits for fodder [6].

Tissue culture derived somaclonal variations are important to create genetic variability and researchers have considered such approach for the selection of suitable somaclones regarding improved crop yield, oil content and stress resistant in peanut [26, 47, 50]. However, there is no reported commercial variety developed through somaclonal selection in peanut. Recently, Wang et al. have reported three peanut varieties with high yield and high oil content, namely Yuhua 4, Yuhua 9, and Yuhua 14 [95]. These varieties have been developed using embryonic leaflets of peanut variety Huayu 20 as explants through *in vitro* mutagenesis. The new peanut varieties contain an oil percentage ranging from 58 to 61%, which is significantly higher than Huayu 20 (49.5%) [95]. Embryonic leaflets culture of irradiated peanut seeds resulted in regenerated peanut plants *in vitro*. The seeds of regenerated plants represented an enhanced oleic acid, linoleic acid, palmitic acid and fat content by 5%, 7%, 3% and 2%; respectively than the mutagenic parent [96]. Mutations in two homoeologs gene sequences of *FAD2A* and *FAD2B*, originated from the genomes of peanut progenitor species *Arachis duranensis* and *Arachis ipaensis*, have been reported for enhanced oleic acid (>70%) content in peanut [97, 98]. Hence, further researches in *FAD2A* and *FAD2B* could be a future rational for high oleic acid peanut development through tissue culture approaches. *AtLEC1* gene is believed to regulate the biosynthesis of lipids in legume seeds. *Agrobacterium* mediated genetic transformation of *AtLEC1* through tissue culture using epicotyl explants represented 4.5-16% increased oil content in seeds of regenerated transgenic peanut plants. Additionally, seeds of the transgenic plants showed high seed weight including enhanced oleic acid, linoleic acid and stearic acid content without causing major changes in agronomic traits [94]. The research attempted *in vitro* targeted mutation in peanut *fatty acid desaturase 2* (*AhFAD2*) using Transcription Activator like

Effector Nucleases (TALENs). It was observed that the mutation frequencies among *AhFAD2* mutant regenerated lines were significantly associated with oleic acid accretion [93]. The ELISA test also confirmed the enriched methionine content in *in vitro* developed transgenic peanut accompanied with high expression of *2S albumin* gene [99]. The peanut allergy is one of the most health hazardous food allergies; extremely reduce the peanut seed quality. An *in vitro Agrobacterium* mediated transgenic approach using peanut hypocotyl explants was used to eliminate the immune dominant allergen *Arah2* protein through RNA interference (RNAi) which is an established natural phenomenon of gene silencing or down regulating specific gene expression [92].

5.2. Improving biotic stress tolerance

Major yield potential could be attained by the development of genotypes tolerant to biotic and abiotic stresses. Peanuts are sensitive to the fungal diseases such as peanut tikka disease (*Cercosporidium personatum*), collar rot (*Aspergillus niger*), rust (*Puccinia arachidis*), late leaf spot (*Phaeoisariopsis personata*), early leaf spot (*Cercospora arachidicola*), stem and pod rot (*Sclerotium rolfsii*), aflaroot or yellow mold (*Aspergillus flavus*) [10, 100]. Tissue culture approaches in combination with the genetic transformation and mutagenesis could be the intriguing aspects for the development of peanut genotypes tolerance to those biotic stresses. The *C. personatum* resistant peanut genotypes were developed through the genetic transformation of *β1–3 glucanase* using embryonic leaflet culture [101]. The genetic transformation of *β1–3 glucanase*, *chitinase*, *AdSGT1*, *CaMV 35 S*, *RsAFP1* and *RsAFP2* using different explants such as cotyledon, cotyledonary node, embryo axes, shoot bud have been reported for transgenic peanut development *in vitro* that showed resistance against rust, early and late leaf spot diseases [102-104]. Gamma radiation was used for mutant development using different explants including leaf, shoot, cotyledon, and hypocotyl for successful regeneration of peanut. The regenerated mutants represented a high resistant to aflatoxigenic fungi (*A. flavus* and *A. parasiticus*) compared to the parents [15]. Mutant development using callus cultures from immature leaf explants of peanut showed resistance to *C. personatum* [105]. Several peanut genotypes have been reported as resistance to a number of viral diseases through genetic transformation using callus and embryonic culture [104, 106]. Peanut productivity and quality are also reduced by the insect infestation, and insects can play a major role as vectors of viral diseases [107]. However, *cry* genes from *Bacillus thuringiensis* have been reported for the development of insect resistance transgenes [107]. The peanut transgenic developed through tissue (cotyledon, shoot and embryo) culture including *Cry1AcF*, *Cry8Ea*, *Cry1EC* and *CryIA(c)* represented resistance against a wide range of Lepidoptera insects [27, 107].

5.3. Improving abiotic stress tolerance

Plants response to abiotic stresses is dependent on the activation and synchronization of stress related genes, which are involved in the biosynthesis of polyamines, trehalose, galactinol and osmolytes such as proline, betaine and glycine which play vital role in plant defense system against abiotic stresses like drought, heat, cold, salinity etc. [20]. Scarcity of water leading to drought and salinity are the prominent abiotic stresses threatening peanut productivity and quality irrespective of peanut growing regions and seasons [28].

Somaclonal selection of *in vitro* regenerated peanut using epicotyl, hypocotyl, and immature leaf culture showed moderate drought tolerant coupled with early maturity and increased shelling percentage in the selected somaclones of peanut variety Sinpadetha 1 mutant [47]. Another research reported repeated cycles of *in vitro* selection as an effective method to produce drought tolerant peanut genotypes with higher proline content [50]. Transgenic peanut developed using *in vitro* culture of a cotyledonary node for a stress-inducible expression of *AtHDG11* ensued enhanced drought and salt tolerance. The regenerated transgenic plants displayed high yield under both salt and drought stresses. Moreover, the plants showed higher free proline content including better water

use efficiency through longer root system, reduced stomatal density, higher chlorophyll content and photosynthetic rates [108]. The *Agrobacterium* mediated genetic transformation of *AtDREB1A* in peanut transgenic plants also showed tolerance to severe soil-moisture deficit without any morphological abnormality [109]. Drought tolerant cell lines of peanut were developed using callus culture under different levels of polyethylene glycol (0, 0.4, 0.6, 0 8 and 1.0 MPa). The selected cells showed higher proline content, soluble amino acids and reducing sugars and gained weight under higher stress levels [25]. The regulated expression of *IPT* in peanut transgenic lines significantly improved drought tolerance in both laboratory and field conditions [110].

The plants regenerated from leaf callus cultures in the MS medium supplemented with 1 mg/l BAP and NAA grew well on salt-amended media containing 0-150 mM NaCl. *In vitro* regenerated plants from salt media were effectively employed to select salt-tolerant somaclones of peanut, including 4-8 folds higher free proline content and significant growth enhancement [17, 49]. The salinity resistance in regenerated peanut transgenic plants was enhanced by the activity of *Pseudomonas fluorescens* strain *TDK1* possessing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which was accompanied by the higher yield in transgenic plants [111]. *AtNHX1* transformed into peanut plants through shoot culture using cotyledon explants displayed increased tolerance to salinity. The transgenic plants exhibited more chlorophyll content, high photosynthetic rate, more biomass production, leading to improved yield and better quality [112]. Over expression of a stress-responsive helicase, *PDH45,* in transgenic peanut using embryonic axes culture showed a superior water retention capacity and fundamental cellular tolerance to drought [113].

In view of the increasing importance of the peanut as a nutrient rich crop, as well as due to emerging climate change, newer challenges are encountered for sustainable peanut cultivation. The *in vitro* success of genetic transformation or mutagenesis in peanut is still inadequate due to appropriate tissue culture protocol, genotypes, explants, and growth environment. Optimization of these factors influencing the *in vitro* regeneration protocols in peanut would feasibly progress the efficiency of transgenic or mutant development related to stress resistance and seed quality over a brief span of time.

6. CONCLUSIONS

Several abiotic stress factors such as salinity, drought, extensively impede peanut production and nutrient content. Moreover, peanut plants can be severely infected by different insects or pathogens in field conditions, which may lead to the use of insecticides or pesticides, reducing the nutritional quality. Moreover, it is challenging and costly to isolate or extract the nutritional or bioactive compounds from the field samples due to their complex physiological and biological reactions. *In vitro* tissue culture allows the plant to grow under a specific environment free from all natural environmental factors or contaminants. The plants regenerated *in vitro* are predicted to be homogenous, however, due to the involvement of intrinsic and extrinsic factors in development under artificial conditions lead to the high probability of genetic and epigenetic changes showing somaclonal variation. Therefore, tissue culture based genetic transformation; mutagenesis and selection of superior somaclones could be the remarkable tools for the dissection of the physiological, biochemical and molecular regulation of peanut plant biology related to development, nutrient content, and stress response phenomena. Thus, future research could focus on enhancing the conversion frequency of somatic embryos after transformation or mutagenic treatment into normal plantlet regeneration with superior stress response or seed quality.

Authors' Contributions: Conceptualization, review collection, original draft preparation and writing by AD; conceptualization, writing, reviewing and editing by AF and reviewing and revising the manuscript by JCG. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest: The author has no conflict of interest to declare.

Acknowledgment: Authors are delighted to acknowledge The World Academy of Sciences (TWAS) and the Swedish International Development Cooperation Agency (Sida) for funding the peanut tissue culture project, on which this review has been apprehended. Authors would like to thank the Department of Genetics and Plant Breeding for providing the laboratory facilities.

Funding: This review has been adopted as a part of research project funded by The World Academy of Sciences (TWAS).

REFERENCES

1. Bakoye O, Baoua I, Sitou L, Moctar MR, Amadou L, Njoroge AW, et al. Peanut production and storage in the sahel: challenges and opportunities in the Maradi and Zinder Regions of Niger. J Agric Sci. 2019; 11(4): 25-34.
2. Pal A, Pal AK. Physiological basis of salt tolerance in peanut (*Arachis hypogaea* L.). Int J Curr Microb Appl Sci. 2017; 6: 2157-2171.
3. FAO (2018) Food Outlook. Retrieved from http://www.fao.org/3/CA0239EN/ca0239en.pdf.
4. Atlas Big (2019) Retrieved from https://www.atlasbig.com/en-in/countries-by-peanut-production.
5. FAO (2019) Retrieved from https://www.nationmaster.com/nmx/ranking/peanuts-production
6. Janila P, Pandey MK, Shasidhar Y, Variath MT, Sriswathi M, Khera P, et al. Molecular breeding for introgression of fatty acid desaturase mutant alleles (ahFAD2A and ahFAD2B) enhances oil quality in high and low oil containing peanut genotypes. Plant Sci. 2016; 242: 203-213.
7. Shokunbi OS, Fayomi ET, Sonuga OS, Tayo GO. Nutrient composition of five varieties of commonly consumed Nigerian peanut (*Arachis hypogaea* L.). Grasas Aceites. 2012; 63: 14-18.
8. Janila P, Nigam SN, Abhishek R, Anil Kumar V, Manohar SS, Venuprasad R. Iron and zinc concentrations in peanut (*Arachis hypogaea* L.) seeds and their relationship with other nutritional and yield parameters. J Agric Sci. 2014; 153(6): 975-994.
9. Arya SS, Salve AR, Chauhan S. Peanuts as functional food: a review. J Food Sci Technol. 2016; 53: 31-41.
10. Kumar V, Thirumalaisamy PP. Diseases of peanut. Disease of field crops and their management. Indian Phytopathological Society, Today and Tomorrow's Printers and Publishers, New Delhi. 2016; 445-494.
11. Coulibaly MA, Ntare P, Gracen Danquah BR, Gracen VE, Kwadwo O. Peanut production constraints and farmers' preferred varieties in Niger. Int J Innov Sci Eng Technol. 2017; 4: 2348-7968.
12. Kambiranda DM, Vasanthaiah HK, Katam R, Ananga A, Basha SM, Naik K. Impact of drought stress on peanut (*Arachis hypogaea* L.) productivity and food safety. Plants Env. 2011; 21: 249-272.
13. Azad MA, Hamid MA, Yasmine F. Enhancing abiotic stress tolerance in peanut through induced mutation. In: Tomlekova NB, Kozgar MI, Wani MR (eds.), Mutagenesis: exploring genetic diversity of crops. 2014; 331-346.
14. Khalifa MM, El-Sayeda HM, El-Badawy MF. Abol-Ela, Gomaa AM. Influence of some biofertilizers and different sources of mineral phosphorus on controlling pod rot diseases and aflatoxin contamination in seeds of peanut. Ann Agric Sci. 2010; 48: 37-48.
15. Azzam C, Khalifa M. Peanut mutants resistant to aflatoxin induced through gamma ray and somaclonal variation and its associated genetic molecular markers. Proceedings of The IRES 26th International Conference, Paris, France. 2016.

16. Waliyar F, Kumar PL, Ntare BR, Monyo E, Nigam SN, Reddy AS, Osiru M, Diallo AT. A century of research on peanut rosette disease and its management. Information Bulletin no. 75. 2007.
17. Dhuha SM, Taghian AS, El-Aref HM, Abd-El-Fatah BES. Gene Expression and *in vitro* Selection for Salinity Tolerance in Peanut. Assiut J Agric Sci. 2014; 45; 67-80.
18. Ahmad N, Khan MR, Shah SH, Zia MA, Hussain I, Muhammad A, Ali GM. An efficient and reproducible tissue culture procedure for callus induction and multiple shoots regeneration in peanut (*Arachis hypogaea* L.). J Anim Plant Sci. 2020; 30: 1540-1547.
19. Ferdausi A, Chang X, Hall A, Jones M. Galanthamine production in tissue culture and metabolomic study on Amaryllidaceae alkaloids in Narcissus pseudonarcissus cv. Carlton. Ind Crops Prod. 2020; 144: 112058.
20. Vinocur B, Altman A. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr Opin Biotechnol. 2005; 16: 123-132.
21. Bhatia S, Dahiya R. Concepts and techniques of plant tissue culture science. Modern Appl Plant Biotech Pharm Sci. 2015; 121-156.
22. Espinosa-Leal CA, Puente-Garza CA, García-Lara S. *In vitro* plant tissue culture: means for production of biological active compounds. Planta. 2018; 248: 1-8.
23. Kaeppler SM, Kaeppler HF, Rhee Y. Epigenetic aspects of somaclonal variation in plants. In: Plant Gene Silencing. Springer, Dordrecht. 2000: 59-68.
24. Trivedi R. Effect of Different Factors During *In Vitro* Growth and Multiplication in Peanut Cultivar 'JL-24'. Proceedings of the National Academy of Sciences, India Section B: Biol Sci. 2016; 86: 131-137.
25. Purushotham MG, Vajranabhaiah SN, Patil VS, Chandrashekhara Reddy P, Prasad GG, Prakash, AH. Development of drought tolerant cell lines in peanut (*Arachis hypogeae* L.) genotypes *in vitro*. Indian J Plant Physiol. 1998; 3: 283-286.
26. Robinson JP, Srivardhini S, Sasikumar G. Somatic embryogenesis and plant regeneration from cotyledon tissue of *Arachis hypogaea* L. Res Plant Biol. 2011; 1.
27. Keshavareddy G, Rohini S, Ramu SV, Sundaresha S, Kumar AR, Kumar PA, Udayakumar M. Transgenics in peanut (*Arachis hypogaea* L.) expressing cry1AcF gene for resistance to *Spodoptera litura* (F.). Physiol Mol Biol Plants. 2013; 19: 343-352.
28. Gantait S, Mondal S. Transgenic approaches for genetic improvement in peanut (*Arachis hypogaea* L.) against major biotic and abiotic stress factors. J Genetic Eng Biotech. 2018; 16: 537-544.
29. Feldman EB. Assorted monounsaturated fatty acids promote healthy hearts. Am J Clin Nutr. 1999; 70: 953-954.
30. USDA (United States Department of Agriculture) (2014): http://www. nal.usda.gov/fnic/foodcomp/search/. Accessed 21 Aug 2014.
31. Morris MC. Dietary niacin and the risk of incident Alzheimer's disease and of cognitive decline. J Neurol Neurosurg Psychiatry. 2004; 75: 1093-1099.
32. Griel AE, Eissenstat B, Juturu V, Hsieh G, Kris-Etherton PM. Improved diet quality with peanut consumption. J Am Coll Nutr. 2004; 23: 660-668.
33. King DE. Dietary magnesium and C-reactive protein levels. J Am Coll Nutr. 2005; 24: 166-171.
34. Song Y, Ridker PM, Manson JE, Cook NR, Buring JE, Liu S. Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. Diabetes Care. 2005; 28: 1438-1444.
35. Coates A, Howe P. Edible nuts and metabolic health. Curr Opin Lipidol. 2019; 18: 25-30.

36. Yu JM, Ahmedna M, Goktepe I, Dai J. Peanut skin procyanidins: composition and antioxidant activities as affected by processing. J Food Compos Anal. 2006; 19: 364-371.
37. Sabate J, Ang Y. Nuts and health outcomes: new epidemiologic evidence. Am J Clin Nutr. 2009; 89: S1643-S1648.
38. Geulein I. Antioxidant properties of resveratrol: a structure activity insight. Innov Food Sci Emerg Technol. 2010; 11: 210-218.
39. Gonzalez CA, Salvado JS. The potential of nuts in the prevention of cancer. Br J Nutr. 2006; 96: S87-S94.
40. Woyengo TA, Ramprasath VR, Jones PJ. Anticancer effects of phytosterols. Eur J Clin Nutr. 2009; 63: 813-820.
41. Fazel Nabavi S, Li H, Daglia M, Nabavi MS. Resveratrol and stroke: from chemistry to medicine. Curr Neurovasc Res. 2014; 11: 390-397.
42. Awad AB, Chan KC, Downie AC, Fink CS. Peanuts as a source of β-sitosterol, a sterol with anticancer properties. Nutr Cancer. 2000; 36: 238-241.
43. Jiang R, Wang M, Davis S. Nut and peanut butter consumption and risk of type 2 diabetes in women. J Am Med Assoc. 2002; 288: 2554-2560.
44. Craft BD, Hargrove JL, Greenspan P, Hartle DK, Amarowicz R, Pegg RB. Recent advances in food and flavor chemistry. Food flavor and encapsulation, health benefits, analytical methods, and molecular biology of functional foods. R Soc Chem. 2010; 283-296.
45. Zhang Y, Zhang H, Wang L, Guo X, Qi X, Qian H. Influence of the degree of hydrolysis (DH) on antioxidant properties and radicalscavenging activities of peanut peptides prepared from fermented peanut meal. Eur Food Res Technol. 2011; 232: 941-950.
46. Debsharma S, Roy K, Ghosh S, Razzak M, Nath U, Azad A. *In vitro* callus development and plant regeneration from immature cotyledons-the best technology for sustainable peanut (*Arachis hypogaea* L.) production in the modern era. Int J Exp Agric. 2020; 10: 26-37.
47. Khin SM, Tin Y, Khine OA, Hla SY, Khin MN, Htun S, Khin S, Tin S. Somaclonal variation of peanut (*Arachis hypogaea* L.) induced by cell culture. J Agric For Lives Fish Sci. 2003; 40-53.
48. Neelakandan AK, Wang K. Recent progress in the understanding of tissue culture-induced genome level changes in plants and potential applications. Plant Cell Rep. 2012; 31: 597-620.
49. Jain M, Mathur G, Koul S, Sarin N. Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of peanut (*Arachis hypogaea* L.). Plant Cell Rep. 2001; 20: 463-468.
50. Hemon AF, Sudarsono. Evaluation of somaclones peanut plants regenerated from repeat cycles of in vitro selection against drought stress. Indonesian J Agron. 2010; 38(1): doi.org/10.24831/jai.v38i1.1677.
51. Gernand D, Golczyk H, Rutten T, Ilnicki T, Houben A, Joachimiak AJ. Tissue culture triggers chromosome alterations, amplification, and transposition of repeat sequences in Allium fistulosum. Genome. 2007; 50: 435-442.
52. Jin S, Mushke R, Zhu H, Tu L, Lin Z, Zhang Y, Zhang X. Detection of somaclonal variation of cotton (*Gossypium hirsutum*) using cytogenetics, flow cytometry and molecular markers. Plant Cell Rep. 2008; 27: 1303-1316.
53. Wu XM, Liu MY, Ge XX, Xu Q, Guo WW. Stage and tissue-specific modulation of ten conserved miRNAs and their targets during somatic embryogenesis of Valencia sweet orange. Planta. 2011; 233: 495-505.
54. Jiang C, Mithani A, Gan X, Belfield EJ, Klingler JP, Zhu J-K, et al. Regenerant Arabidopsis lineages display a distinct genome-wide spectrum of mutations conferring variant phenotypes. Curr Biol. 2011; 21: 1385-1390.

55. Yu X, Li X, Zhao X, Jiang L, Miao G, Pang J, et al. Tissue culture-induced genomic alteration in maize (Zea mays) inbred lines and F1 hybrids. Ann Appl Biol. 2011; 158: 237-247.
56. Linacero RE, Alves F, Vazquez AM. Hot spots of DNA instability revealed through the study of somaclonal variation. Theor Appl Genet. 2000; 100: 506-511.
57. Miguel C, Marum L. An epigenetic view of plant cells cultured *in vitro*: somaclonal variation and beyond. J Exp Bot. 2011; 62(11): 3713-3725.
58. Madlung A, Comai L. The effect of stress on genome regulation and structure. Ann Bot. 2004; 94: 481-495.
59. Palanivel S, Parvathi S, Jayabalan N. *In vitro* culture of mature embryo axes of peanut (*Arachis hypogaea* L.) J Indian Bot Soc. 2001; 80: 15-19.
60. Palanivel S, Jayabalan N. Direct multiple shoot induction from different mature seed explants of peanut (*Arachis hypogaa* L.) Philippines J Sci. 2002; 131: 127-131.
61. Iqbal MM, Nazir F, Iqbal J, Tehrim S, Zafar Y. *In vitro* micropropagation of peanut (*Arachis hypogaea*) through direct somatic embryogenesis and callus culture. Int J Agric Biol. 2011; 13: 811-814.
62. Ozias-Akins P, Gill R. Progress in the development of tissue culture and transformation methods applicable to the production of transgenic peanut. Peanut Sci. 2001; 28: 123-131.
63. Geng L, Niu L, Shu C, Song F, Huang D, Zhang J. High-efficiency regeneration of peanut (*Arachis hypogaea* L.) plants from leaf discs. Afr J Biotech. 2011; 10: 12650-12652.
64. Nazir F, ulHasan M, Akram Z, Javed MM, Ali S, Ali GM, Zafar Y. *In vitro* regeneration of Pakisthani peanut (*Arachis hypogaea* L.) varieties using de-embryonated coteledonary explants. Afr J Biotech. 2011; 10: 8599-8604.
65. Arockiasamy S, Prakash S, Ignacimuthu S. Direct organogenesis from mature leaf and petiole explants of *Eryngium foetidum* L. Biol Plant. 2002; 45: 129-132.
66. Venkatachalam P, Kavipriya V. Efficient method for *in vitro* plant regeneration from cotyledonary node explants of peanut (*Arachis hypogaea* L.). International Conference on Nuclear Energy, Environmental and Biological Sciences (ICNEEBS'2012). 2012: 8-9.
67. Ozudogru EA, Kaya E, Lambardi M. *In vitro* propagation of peanut (*Arachis hypogaea* L.) by shoot tip culture. In Protocols for Micropropagation of Selected Economically-Important Horticultural Plants. Humana Press, Totowa, NJ. 2012: 77-87.
68. Krishna G, Singh BK, Kim EK, Morya VK, Ramteke PW. Progress in genetic engineering of peanut (*Arachis hypogaea* L.). A review. Plant Biotech J. 2015; 2: 147-162.
69. Adu-Dapaah HK, Sangwan RS. Improving bambara peanut productivity using gamma irradiation and *in vitro* techniques. Afr J Biotech. 2004; 3: 260-265.
70. Koné M, Koné T, Silué N, Soumahoro AB, Kouakou TH. *In vitro* Seeds Germination and Seedling Growth of Bambara Peanut (*Vigna subterranea* (L.) Verdc. (Fabaceae). Sci World J. 2015: 595073.
71. Katembe WJ, Ungar IA, Mitchell JP. Effect of salinity on germination and seedling growth of twoAtriplexspecies (Chenopodiaceae). Ann Bot. 1998; 82: 167-175.
72. Baskin CC, Baskin JM. Germination ecophysiology of herbaceous plant species in a temperate region. Am J Bot. 1988; 75: 286-305.
73. Ishag S, Osman MG, Khalafalla MM. Effects of growth regulators, explant and genotype on shoot regeneration in tomato (Lycopersicon esculentum cv Omdurman). Int J Sustain Crop Prod. 2009; 4: 7-13.
74. Tiwari S, Rakesh T. Multiple shoot regeneration in seed-derived immature leaflet explants of peanut (*Arachis hypogaea* L.). Sci Hortic. 2009; 121: 223-227.

75. Shan L, Tang G, Xu P, Liu Z, Bi Y. High efficiency *in vitro* plant regeneration from epicotyl explants of Chinese peanut cultivars. *In Vitro* Cell Dev Biol-Plant. 2009; 45: 525.
76. Alam AK, Khaleque MA. *In vitro* response of different explants on callus development and plant regeneration in peanut (*Arachis hypogeae* L.). Int J Exp Agric. 2010; 1: 1-4.
77. Venkatachalam P, Geetha N, Khandelwal A, Shaila MS, Sita GL. Agrobacterium-mediated genetic transformation and regeneration of transgenic plants from cotyledon explants of peanut (*Arachis hypogaea* L.) via somatic embryogenesis. Curr Sci. 2000; 78: 1130-1136.
78. Hussain KM, Anis M, Shahzad A. *In vitro* propagation of a multipurpose leguminous tree (*Pterocarpus marsupium* Roxb.) using nodal explants. Acta Physiol Plant. 2008; 30: 353-359.
79. Perveen S, Varsney A, Anis M, Aref IM. Influence of cytokinins, basal media and pH on adventitious shoot regeneration from excised root cultures of Albizia lebbeck. J Forestry Res. 2011; 22: 47-52.
80. Ismail N, Rani U, Batra A. High frequency *in vitro* regeneration of Clitoria ternatea L. affected by different cultural conditions. Ind J Biotech. 2012; 11: 210-214.
81. Yaseen M, Ahmad T, Sablok G, Standardi A, Hafiz IA. Review: role of carbon sources for *in vitro* plant growth and development. Mol Biol Rep. 2013; 40: 2837-2849.
82. George EF, Hall MA, De Klerk GJ. Plant growth regulators I: introduction; auxins, their analogues and inhibitors; Plant growth regulators II: cytokinins, their analogues and antagonists. Plant propagation by tissue culture. Springer, 2008.
83. Nagori R, Purohit SD. *In vitro* plantlet regeneration in Annona squamosa through direct shoots bud differentiation on hypocotyls segments. Sci Hortic. 2004; 99: 89-98.
84. Aasim M, Khawar KM, Ozcan S. *In vitro* micropropagation from plumular apices of Turkish cowpea (*Vigna unguiculata* L.) cultivar Akkiz. Scientia Hortic. 2009; 122: 468-471.
85. Guo B, Abbasi BH, Amir Zeb, Xu LL, Wei YH. Thidiazuron: A multi-dimensional plant growth regulator. Afr J Biotechnol. 2011; 10: 8984-9000.
86. Kakani A, Li G, Peng Z. Role of AUX1 in the control of organ identity during *in vitro* organogenesis and in mediating tissue specific auxin and cytokinin interaction in Arabidopsis. Planta. 2009; 229(3): 645-657.
87. Bhanumathi P, Ganesan M, Jayabalan N. A simple and improved protocol for direct and indirect somatic embryogenesis of peanut (*Arachis hypogaea* L.). J Agric Technol. 2005; 1: 327-344.
88. Cucco MF, Rossi Jaume AD. Protocol for regeneration *in vitro* of *Arachis hypogaea* L. Electron J Biotechnol. 2000; 3: 35-50.
89. Akasaka YH, Daimon, Mii M. Improved plant regeneration from cultured leaf segments in peanut (*Arachis hypogaea* L.) by limited exposure to thidia-zuron. Plant Sci. 2000; 156: 169-175.
90. Verma A. Response of peanut varieties to plant growth regulator (BAP) to induce direct organogenesis. World J Agric Sci. 2009; 5: 313-317.
91. Masanga J, Ommeh S, Kasili R, Alakonya A. An optimized protocol for high frequency regeneration of selected peanut (*Arachis hypogaea* L.) varieties from East Africa using cotyledons. Intl J Agri Crop Sci. 2013; 6(20): 1421-1425.
92. Dodo HW, Konan KN, Chen FC, Egnin M, Viquez OM. Alleviating peanut allergy using genetic engineering: the silencing of the immunodominant allergen Ara h 2 leads to its significant reduction and a decrease in peanut allergenicity. Plant Biotech J. 2008; 6: 135-145.

93. Wen S, Liu H, Li X, Chen X, Hong Y, Li H, Lu Q, Liang X. TALEN-mediated targeted mutagenesis of fatty acid desaturase 2 (FAD2) in peanut (*Arachis hypogaea* L.) promotes the accumulation of oleic acid. Plant Mol Biol. 2018; 97: 177-185.
94. Tang G, Xu P, Ma W, Wang F, Liu Z, Wan S, Shan L. Seed-specific expression of atlec1 increased oil content and altered fatty acid composition in seeds of peanut (*Arachis hypogaea* l.). Front Plant Sci. 2018; 9: 260.
95. Wang JS, Lei SH, Yue LI, Zhao MX, Xia WA, Qiao LX, et al. Development of peanut varieties with high oil content by in vitro mutagenesis and screening. J Int Agric. 2020; 19(12): 2974-2982.
96. Wang JS, Qiao LX, Zhao LS, Wang P, Guo BT, Liu LX, Sui JM. Performance of peanut mutants and their offspring generated from mixed high-energy particle field radiation and tissue culture. Genet Mol Res. 2015; 14(3): 10837-10848.
97. Bertioli DJ, Jenkins J, Clevenger J, Dudchenko O, Gao D, Seijo G, et al. The genome sequence of segmental allotetraploid peanut *Arachis hypogaea*. Nat Genetics. 2019; 51: 877-884.
98. Ojiewo CO, Janila P, Bhatnagar-Mathur P, Pandey MK, Desmae H, Okori P, et al. Advances in crop improvement and delivery research for nutritional quality and health benefits of peanut (*Arachis hypogaea* L.). Front Plant Sci. 2020; 11: 29.
99. Lacorte C, Aragao FJ, Almeida ER, Rech EL, Mansur E. Transient expression of GUS and the 2S albumin gene from Brazil nut in peanut (*Arachis hypogaea* L.) seed explants using particle bombardment. Plant Cell Rep. 1997; 16(9): 619-623.
100. Sunkara S, Bhatnagar-Mathur P, Sharma KK. Transgenic interventions in peanut crop improvement: progress and prospects. Gen Genom Breed Peanuts. 2014; 179-216.
101. Qiao LX, Ding X, Wang HC, Sui JM, Wang JS. Characterization of the beta-1,3-glucanase gene in peanut (*Arachis hypogaea* L.) by cloning and genetic transformation. Genet Mol Res. 2014; 13: 1893-1904.
102. Iqbal MM, Nazir F, Ali S, Asif MA, Zafar Y, Iqbal J, Ali GM. Over expression of rice chitinase gene in transgenic peanut (*Arachis hypogaea* L.) improves resistance against leaf spot. Mol Biotechnol. 2012; 50: 129-136.
103. Prasad K, Bhatnagar-Mathur P, Waliyar F, Sharma KK. Overexpression of a chitinase gene in transgenic peanut confers enhanced resistance to major soil borne and foliar fungal pathogens. J Plant Biochem Biotechnol. 2013; 22: 222-233.
104. Mallikarjuna G, Rao TS, Kirti PB. Genetic engineering for peanut improvement: current status and prospects. PCTOC. 2016; 125(3): 399-416.
105. Venkatachalam P, Jayabalan N. Selection and regeneration of peanut plants resistant to the pathotoxic culture filtrate of Cercosporidium personaturn through tissue culture technology. Appl Biochem Biotech. 1996; 61: 351-364.
106. Rao SC, Bhatnagar-Mathur P, Kumar PL, Reddy AS, Sharma KK. Pathogen-derived resistance using a viral nucleocapsid gene confers only partial non-durable protection in peanut against peanut bud necrosis virus. Arch Virol. 2013; 158: 133-143.
107. Geng L, Niu L, Gresshoff PM, Shu C, Song F, Huang D, Zhang J. Efficient production of *Agrobacterium* rhizogenes-transformed roots and composite plants in peanut (*Arachis hypogaea* L.). PCTOC. 2012; 109: 491-500.

108. Banavath JN, Chakradhar T, Pandit V, Konduru S, Guduru KK, Akila CS, et al. Stress inducible overexpression of AtHDG11 leads to improved drought and salt stress tolerance in peanut (*Arachis hypogaea* L.). Front Chem. 2018; 6: 34.
109. Sarkar T, Thankappan R, Kumar A, Mishra GP, Dobaria JR. Stress inducible expression of AtDREB1A transcription factor in transgenic peanut (*Arachis hypogaea* L.) conferred tolerance to soil-moisture deficit stress. Front. Plant Sci. 2016; 7: 935.
110. Qin H, Gu Q, Zhang J, Sun L, Kuppu S, Zhang Y, et al. Regulated expression of an isopentenyltransferase gene (IPT) in peanut significantly improves drought tolerance and increases yield under field conditions. Plant Cell Physiol. 2011; 52: 1904-1914.
111. Saravanakumar D, Samiyappan R. ACC deaminase from Pseudomonas fluorescens mediated saline resistance in peanut (*Arachis hypogea*) plants. J Appl Microbiol. 2007; 102: 1283-1292.
112. Banjara M, Zhu L, Shen G, Payton P, Zhang H. Expression of an Arabidopsis sodium/proton antiporter gene (AtNHX1) in peanut to improve salt tolerance. Plant Biotech Rep. 2012; 6: 59-67.
113. Manjulatha M, Sreevathsa R, Kumar AM, Sudhakar C, Prasad TG, Tuteja N, Udayakumar M. Overexpression of a pea DNA helicase (PDH45) in peanut (*Arachis hypogaea* L.) confers improvement of cellular level tolerance and productivity under drought stress. Mol Biotech. 2014; 56(2): 111-125.
114. Elia FM, Hosfield GL, Kelly JD, Uebersax MA. Genetic analysis and interrelationships between traits for cooking time, water absorption, and protein and tannin content of Andean dry beans. J Am Soc Hort Sci. 1997; 122(4): 512-518.
115. Jambunathan R. Peanut quality characteristics. Uses of Tropical Grain Legumes: proceedings of a Consultative Meeting. 1991: 267-275.
116. Bhuiyan MSA, Haque MM, Hoque MI, Sarker RH, Islam AS. Morphogenic responses of peanut leaflet explaints cultured in vitro. Plant Tissue Cult. 1992; 2(1): 49-53.
117. Radhakrishnan T, Murthy TGK, Chandran K, Bandyopadhyay A. Micro-progagation in peanut (*Arachis hypogaea* L.). Biologia-Plantrum. 2000; 43(3): 447-450.
118. Eapen S, George L. Somatic embryogenesis in peanut: influence of growth regulators and sugars. PCTOC. 1993; 35(2): 151-156.
119. Srinivasan T, Kumar K, Kirti P. Establishment of efficient and rapid regeneration system for some diploid wild species of *Arachis*. PCTOC. 2010; 101: 303-309.
120. Zhao MX, Qiao LX, Sui JM, Tan LL. An efficient regeneration system for peanut: somatic embryogenesis from embryonic leaflets. J Food Agric Envir. 2012; 10: 527-531.